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Leaf Spots of Ornamental Foliage Plants in Egypt with Special Reference to *Corynespora cassiicola* [(Berk. & Curt.)Wei] as a New Causal

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Nine foliage plants grown in greenhouses of Giza governorate were examined during 1999 and 2000 seasons in order to search for leaf spot diseases infection. Twelve fungal species were isolated from tested plants, where the occurrence of each one varied from one plant to another. *Rhizoctonia solani*, *Corynespora cassiicola*, *Alternaria panax*, *Colletotrichum* sp. and *Fusarium moniliforme* were the most dominant fungi in isolation, followed by *Myrothecium roridum*. On the other hand, highest number of fungal species in isolation trials were obtained from only four hosts, *i.e. Dracaena marginata*, *Epipremnum aureum*, *Philodendron cordatum* and *Syngonium podophyllum*. Also, the highest fungal frequency (43.6%) was recorded by *C. cassiicola* on *Dieffenbachia seguine* as well as *A. panax* (44.9%) and *R. solani* (46.4%) on *Schefflera actinophylla*. On the other hand, length and width of conidiophores and conidia of *C. cassiicola* isolates as well as septa / conidium were found to vary according to their original host plant. Pathogenicity and cross-inoculation tests confirmed that all isolates of *C. cassiicola* were pathogenic to *Dracaena marginata*, *Dieffenbachia seguine*, *Epipremnum aureum* and *Syngonium podophyllum*, recording the highest disease indexes on its plant source. On the other hand, Corynespora leaf spot symptoms vary depending upon the host and its lesions, firstly appear as tiny, sunken, slightly brown areas. These areas may enlarge to 2.5 cm in diameter and darken with age. Also, they might expand rapidly to reach 5cm or more, when environmental conditions are favorable. Identification of the four tested isolates as *C. cassiicola* was confirmed by the use of electrophoretic protein patterns which showed 89.72% similarity among isolates forming two main clusters, each consisted of two particular isolates. The first cluster included isolates 3 and 4 isolated from *Syngonium podophyllum* and *Dieffenbachia seguine* with 97.8% similarity while the other one recorded 94.3% similarity between isolates 1 and 2 isolated from *Epipremnum aureum* and *Dracaena marginata*, respectively. On the other hand, the reaction of 10 foliage plants to the four tested isolates of *C. cassiicola* by using detached leaf technique revealed that they greatly vary in this respect. *C. cassiicola* isolate of *Dracaena marginata*, failed to infect any plant species tested. While, only 5 reactions from 40 were positive in the case of isolates of *Dieffenbachia seguine* and *Epipremnum aureum* on *F. benjamina*, *Ficus microcarpa nitida* (cv. Hawaii) and that of *Syngonium podophyllum* on only *Cissus rhombifolia*. No symptoms were

observed by the four isolates of *C. cassiicola* on *Aglaonema commutatum, Asparagus densiflorus, Codiaeum variegatum, Philodendron cordatum, Schefflera actinophylla, Yucca elephantipes* and *Zamioculcas zamiifolia.*

Key words: *Alternaria panax, Colletotrichum* sp., *Corynespora cassiicola, Dracaena marginata, Epipremnum aureum, Fusarium moniliforme,* leaf spot, *Myrothecium roridum, Philodendron cordatum Rhizoctonia solani* and *Syngonium podophyllum.*

Foliage ornamental plants are important tool for modern indoor decoration, but their values and appearance are usually affected by several diseases including leaf spots which are more prevalent, particularly in warm and moist conditions where the disease can lead to heavy defoliation (Knauss *et al.*, 1981; Blessington and Collins, 1993; Chase, 1992 and 1997 and Chase *et al.*, 1995). Corynespora leaf spot caused by the cosmopolitan fungus *C. cassiicola* (Berk. & Curt.) Wei. was consistently associated with a serious disease of many ornamental plants.

The first report of the disease on a foliage plant, however, was mentioned in 1973 on Zebra plant (*Aphelandra squarrosa*) by McRitchie and Miller (1973). Since that time, the fungus has been identified as the cause of the disease on more than 50 ornamental plants (Chase, 1982, 1984 and 1997 and Chase *et al.*, 1995). Woody ornamentals including Azalea, Hydrangea and Ligustrum have also been found to be infected by this pathogen over the past 20 years (Sobers, 1966 and Miller and Alfieri, 1974). Moreover, the fungus attacked a wide range of economic plants as vegetables (cucumber, egg plant, okra, squash, tomatoes, etc.) and field crops (cotton, soybean, sesame, etc.), affecting the leaves, stems, pods, seeds, hypocotyls and roots (Ellis, 1957; Ellis and Holliday, 1971 and Farr *et al.*, 1989).

In this study, Corynespora leaf spot was reported in Egypt during 1999 and 2000 season on five foliage ornamental plants for the first time. Symptoms, however, vary depending upon the infected host, but they firstly appear as tiny, sunken and slightly brown areas. The disease leads to severe defoliation under its environmental favorable conditions in greenhouses. There was very little information about leaf spots of ornamentals in Egypt not including foliage plants (El- Shinnawy, 1964; Ali *et al.*, 1972 and Hilal and Kamel, 1990) as well as on dracaena (Hilal *et al.*, 2000) and the genetic variability in isolates of *C. cassiicola* in literature (Silva *et al.*, 1995; Silva *et al.*, 1998; Saha *et al.*, 2000 and Silva *et al.*, 2003).

Therefore, the present investigation aimed to survey leaf spot diseases on foliage plants and identifying their causal pathogens. Pathogenic capability of *Corynespora cassiicola* isolates, disease symptoms and their hosts, morphological features and protein patterns of four isolates were also investigated.

Materials and Methods

Unless otherwise mentioned, fourteen ornamental foliage plants (Table 1) belonging to seven families were used throughout this study. The natural occurrence of leaf spot infection was surveyed on 9 of them while 10 foliage plants were used in host range test.

Table 1. Ornamental foliage plants tested; family, scientific, cultivar and common names

Family name*	Scientific name and cultivar	Common name*
Agavaceae	*Dracaena marginata* Lam.	Dracaena
	Yucca elephantipes Regel	Spineless Yucca
Araceae	*Aglaonema commutatum* Schott (cv. Silver queen)	Aglaonema
	Dieffenbachia seguine (Jacq.) Schott "Tropic snow"	Dieffenbachia
	Epipremnum aureum (cv. Lindenex andre) Bunt.	Pothos
	Philodendron cordatum (Vell.) Kunth	Philodendron
	Syngonium podophyllum Schott	Nephthytis
	Zamioculcas zamiifolius (Lodd.) Engl.	Zamioculcas
Araliaceae	*Codiaeum variegatum* (L.) Blume (cv. Norma)	Croton
Euphorbiaceae	*Schefflera actinophylla* (Endl.) Jaeger	Umbrella tree
Liliaceae	*Asparagus densiflorus* (Kunth) Jessop (cv. Sprengeri)	Asparagus fern
Moraceae	*Ficus benjamina* L.	Weeping fig
	Ficus microcarpa nitida (cv. Hawaii)	Ficus
Vitaceae	*Cissus rombifolia* Vahl.	Grape ivy

* Names according to Farr *et al.* (1989), Chase (1992) and Blessington and Collins (1993).

1- Survey, isolation and identification of the causal pathogen:

Leaf spot diseases survey was conducted during 1999 and 2000 seasons in several greenhouses and nurseries in Giza governorate on nine species of foliage ornamental plants. Specimens of the diseased plants were taken into the laboratory to isolate the causal pathogens.

For isolation, infected leaf lesions were cut into small fragments (2x2 mm), washed thoroughly with tap water and superficially sterilized with 0.5% sodium hypochlorite for 2 min. Then, the fragments were rinsed several times in sterilized distilled water, blotted to dry on sterile filter paper. Then placed onto PDA plates and incubated at room temperature (26±2°C) for 7 days.

The fungal growth was examined microscopically and purified using the single spore and/or hyphal tip techniques (Dhingra and Sinclair, 1985). Colony characteristics, spore morphology were described and identified. *Corynespora cassiicola* as one of the most frequent causal pathogens was identified and verified by the Mycol. Centre, Assiut Univ., Assiut, Egypt. Whereas, the other fungal isolates were identified by the staff of Mycol. and Dis. Survey Dept., Plant Pathol. Res. Instit., ARC, Giza, Egypt.

2- Pathogenicity tests:

Pathogenicity tests were conducted for only four selected *Corynespora cassiicola* isolates against dracaena, dieffenbachia, nephthytis and pothos in order to investigate the possible specificity within isolates.

Each isolate was grown on PDA plates and incubated at 28°C for 10 days under continuous fluorescent light, which enhance sporulation.

Spore suspension was prepared by adding 10 ml of distilled water to each plate and remove the spores gently using a camel hair brush. The spore suspension was filtered through two layers of cheesecloth into a sterile flask for each isolate. The spore density was adjusted to 7.5 X 10^4 conidia /ml water. Tween 20 (*ca* 0.02% v/v) was added as a wetting agent.

The spore suspension was sprayed on both leaf surfaces of the plant (6-month-old) with an atomizer. Immediately after inoculation, plants were kept at high humidity in polyethylene bags at (28-30°C) for 48 hours. Control plants were similarly treated with only sterile distilled water. Five plants were used for each treatment. The experimental design was split plots, where the 4 tested plants were the main plots and the different isolates were subplots with 4 randomized replicates (pots). Each pot included an individual plant.

Lesions were observed for 4 to 6 days after inoculation. Disease readings were determined for each leaf within 14 days following inoculation according to the disease severity rating which was made to include the size and frequency of the lesion /leaf. The following numerical rates were suggested for disease severity:

0= No symptoms.
1= Few scattered lesions covering about 1-25% of the leaf.
2= Spots covering about 25-50% of the leaf.
3= Spots coalescing and covering about 50-75% of the leaf.
4= Severe infection with coalescing lesion covering more than 75% of the leaf.

Disease index was calculated according to the equation suggested by Townsend and Heuberger (1943) as follows:

$$\text{Disease index (\%)} = \frac{\text{sum } (n \times r)}{4N} \times 100$$

Whereas: (n) is the number of leaves in each numerical grade (r) and (N) is the total number of inoculated leaves multiplied by the maximum numerical grade 4.

3-Host range:

The host range was conducted for four *Corynespora cassiicola* isolates against ten foliage plants, *i.e. Aglaonema commutatum, Asparagus densiflorus, Cissus rhombifolia, Codiaeum variegatum, Ficus benjamina, Ficus microcarpa nitida* (cv. Hawaii), *Philodendron cordatum, Schefflera actinophylla, Yucca elephantipes* and *Zamioculcas zamiifolia.*

A method of Kohpina *et al.* (2000) was employed for testing detached leaves by using Oasis LC-1 Horticubes (Smithers-Oasis Australia Pty Ltd, Elizabeth West, South Australia), which is designed to drain excess water from the base of a cutting while providing the proper balance of water and air, were used as the medium for holding detached leaves. Experiments were conducted in plastic boxes. The Oasis medium was wetted with water containing 5 ppm gibberellic acid according to Burdon and Marshall (1981), before the leaves were planted. In this medium, the leaves remained visibly fresh for up to 30 days which allowed sufficient time for symptom expression. The main stem or the ramification was cut from the top, which

carry five to ten leaves, the big or lobate leaves were excised from the main stem as replicate and immediately immersed into the Oasis medium, then the leaves was sprayed with the spore suspension at $7.5x10^4$ conidia/ml. Boxes were firmly covered with a transparent plastic sheet to maintain humidity and thus enhance spore germination and then kept at 28°C in a glasshouse. Two days after inoculation, the plastic cover was loosened to allow some gas exchange while maintaining high humidity throughout the growth period. Four replicates (leaves) from each host plant were inoculated with each isolate and four replicates were used as control (without inoculation). On the other hand, disease reactions were determined for each leaf within 10 days after inoculation as follows: no infection (-), slight infection (+), moderate infection (++) and severe infection (+++).

4- Molecular variation:

Variation within the tested isolates was investigated by using electrophoresis technique to separate patterns of protein on polyacrylamide gels. Procedures of extraction of mycelial proteins used as described by Khalil *et al.* (1997).

Protein determination:

The concentration of protein present in the samples was estimated by the method reported by Bradford (1976).

SDS-polyacrylamide gel electrophoresis:

Electrophoresis of total protein was carried out on a vertical gradient gel form (5-15% polyacrylamide gels) containing sodium-dodecyl-sulfate (SDS) according to the method of Laemmli (1970). The separating gel composition was acrylamide and N- methylene bisoacrylamide 30:0.8 and tris HCl buffer (pH 8.8). The running buffer composed of 0.025 M-tris and 0.129 M glycine, pH 6.3 and 10% SDS. Each track was loaded with 20µl of protein sample and subsequently run at 2 mA.

Staining of the protein bands and densitometer scanning:

The protein bands were visualized by silver staining method described by Giulian *et al.* (1983). After staining, the gels were washed with glass distilled water and stored in 7% acetic acid until they were photographed. Then they were dried using a gel drier and scanned in a recording gel densitometer (Gs 365 w-Hoefer scientific instruments). A gel documentation system was used then to cluster the protein patterns.

Results

1- Survey, isolation and identification of the causal pathogens of the leaf spot diseases:

Data in Table (2) indicate that twelve fungal species were isolated from leaf spot diseases of nine foliage tested plants. However, the occurrence and frequency of each of the isolated fungi varied from one plant to another. *Rhizoctonia solani* (21.1%), *Corynespora cassiicola* (16.4%), *Alternaria panax* (16.0%), *Colletotrichum* sp. (12.9%) and *Fusarium moniliforme*(12.9%), were the most dominant fungi in isolation trials, since they gave the highest frequencies, followed by *Myrothecium roridum* (9.0%). Also, they were isolated from 5 to 7 plants from

Table 2. Survey of fungi associated with leaf spots of nine foliage plants grown in greenhouses at Giza governorate and their frequency in isolation trials

Fungus	Frequency (%) on;*									
	Ag	Cr	Di	Dr	Gr	Ne	Ph	Po	Um	Mean
Alternaria panax	0.0	37.1	0.0	21.1	0.0	22.6	0.0	18.0	44.9	16.0
Asteromella sp.	0.0	0.0	0.0	16.4	0.0	0.0	0.0	0.0	0.0	1.8
Botryodiplodia spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.0	0.0	1.6
Cephalosporium cinnamomeum	0.0	0.0	30.9	0.0	0.0	0.0	0.0	0.0	0.0	3.4
Colletotrichum sp.	32.5	0.0	0.0	20.5	33.3	0.0	20.8	0.0	8.7	12.9
Corynespora cassiicola	0.0	0.0	43.6	25.7	34.6	23.2	0.0	20.3	0.0	16.4
Cylindrocarpon sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.5	0.0	1.5
Dactylaria humicola	0.0	0.0	0.0	0.0	0.0	0.0	17.0	0.0	0.0	1.9
Fusarium moniliforme	0.0	36.2	0.0	16.4	0.0	22.0	23.3	18.0	0.0	12.9
Myrothecium roridum	33.8	0.0	25.5	0.0	0.0	0.0	22.0	0.0	0.0	9.0
Nigrospora sp.	0.0	0.0	0.0	0.0	0.0	14.1	0.0	0.0	0.0	1.6
Rhizoctonia solani	33.8	26.7	0.0	0.0	32.1	18.1	17.0	16.2	46.4	21.1

* Frequency (%) on: Ag= Aglaonema, Cr= Croton, Di= Dieffenbachia, Dr= dracaena, Gr= Grape Ivy, Ne= Nephthytis, Ph= Philodendron, Po= Pothos and Um= Umbrella tree

the nine experimented ones. While, the rest six fungi were the least in this respect, since they gave 1.5%-3.4 % frequencies and only one plant was infected by each of the isolated fungi. On the other hand, dracaena, nephthytis, philodendron and pothos were found to be the most susceptible plants to the fungal species tested. In this respect, six fungi were isolated from pothos and five ones were isolated from the others. Also, the highest frequency percentages were recorded by *C. cassiicola* (43.6%) on dieffenbachia and *A. panax* (44.9%) and *R. solani* (46.4%) on umbrella tree. Whereas, *Colletotrichum* sp. recorded the least frequency percentage (8.7%) on umbrella tree.

2- Corynespora leaf spot symptoms on the plants surveyed under natural infection:

Corynespora leaf spot of ornamental foliage plants tested were usually a problem during the propagation phase, since the plants were kept under very high moisture and humidity conditions. Spots started on lower leaves, especially those wounded and /or in contact with the potting medium. Symptoms of the disease varied depending upon the host and appeared on one or both leaf surfaces. Lesions firstly appear as tiny, sunken, slightly brown areas. These areas always enlarge to about 2.5 cm in diameter and darken with age and often appeared wet. Lesions expanded rapidly and might reach 5 cm or more, when environmental conditions are favorable. A dull green or yellowish green halo commonly surrounds the lesions, which often become concentrically ringed at maturity.

3–The morphology of Corynespora cassiicola isolates:

All the isolates of Corynespora were identified as *Corynespora cassiicola* (Berk. & Curt.) Wei. by the staff of the Mycological Centre, Assiut Univ., Egypt,

depending upon morphotaxonomic features. The mycelium is immersed, with no stroma. Conidiophores were pale to light brown conidiophores, multiseptate with 9 successive proliferations, 4-11 µm wide and 110-850 µm long, produced singly or in clusters, (Fig. 1).

Fig. 1: Conidiophores of *Corynespora cassiicola* (400x).

Conidia developed only at the conidiophore apex, large or small conidia in chains, but each apex may be succeeded by an additional one as the conidiophore proliferates through the opening occupied by a shed of conidia. In this manner, by percurrent proliferation, one or more cylindrical, apical extensions develop each successive apex produce a conidium. Conidia, however, are smoky to olivaceous, smooth, obclavate to cylindrical, subhyaline to pale olivaceous brown, 4-20 pseudoseptete, the size range is 40-220 x 9-22 µm (Fig.2).

Fig. 2. Conidia of *Corynespora cassiicola* (400x).

On the other hand, it was evident from the data presented in Table (3) that there is size variation among *C. cassiicola* isolates. Length of conidiophores and conidia in nephthytis isolate were much longer than in other isolates, since they were 710.4 and 177.8 µm (as mean of 100 readings), respectively. While, in dieffenbachia isolate the length was much shorter (441.6 and 110.4 µm) than in other isolates. The number of pseudosepta in conidia taken from different isolates were 7.0 to 11.0. While, the width of conidia were 12.2 to 12.8 µm and the width of conidiophores ranged from 6.0 to 6.2 µm.

Table 3. Comparison of morphotaxonomic features of the four *Corynespora cassiicola* isolates

Source of isolate	Conidiophores		Conidia		
	Average length (µm)	Average width (µm)	Average length (µm)	Average width (µm)	Mean No. of septa/conidium
Dieffenbachia	441.6	6.0	110.4	12.2	8.0
Dracaena	494.8	6.1	123.8	12.4	8.0
Nephthytis	710.4	6.0	177.8	12.2	11.0
Pothos	450.2	6.2	112.6	12.8	7.0

4-Pathogenicity tests using the four C. cassiicola isolates:

Four selected foliage ornamental plants, *i.e.* dieffenbachia, dracaena, nephthytis and pothos were infected by all tested isolates of *C. cassiicola* Table (4) and Figs. (3, 4 and 5). In all cases, dracaena isolate was the most virulent, (35.60%). While, dieffenbachia isolate was the least aggressive (29.08%). Percentages of the disease index revealed significant differences in the interaction between the tested plants and *C. cassiicola* isolates. The highest disease index was always recorded on a host plant inoculated by its fungal isolate. Thus, isolates of dieffenbachia, dracaena, nephthytis and pothos gave disease indexes by 33.0%, 48.87%, 48.21% and 42.33% on their respective hosts. However, the lowest significant disease index on dracaena (25.0%), nephthytis (26.38%) and pothos (29.16%) were recorded by *C. cassiicola* isolated from dieffenbachia, pothos and nephthytis, respectively. On the other hand, isolates of C. *cassiicola* isolated from dracaena, nephthytis and pothos were similar and gave the same disease index (25.0%) when inoculated on leaves of dieffenbachia.

Table 4. Pathogenicity test of four *C. cassiicola* isolates on four foliage plants and cross inoculation, 14 days after inoculation under greenhouse conditions

Source of isolate	Disease index (%) on different hosts				Mean
	Dieffenbachia	Dracaena	Nephthytis	Pothos	
Dieffenbachia	33.00	25.00	29.16	29.16	29.08
Dracaena	25.00	48.87	26.66	41.87	35.60
Nephthytis	25.00	29.16	48.21	25.00	31.84
Pothos	25.00	26.50	26.38	42.33	30.05
Mean	27.00	32.38	32.60	34.59	
L.S.D. at 5% for: Isolates (I)= 0.7; Plants (P)= 0.7 and I X P = 1.4					

Fig. 3. Lesions caused by *Corynespora cassiicola* on leaves of syngonium (nephthytis).

Fig. 4. Lesions caused by *Corynespora cassiicola* on leaves of pothos.

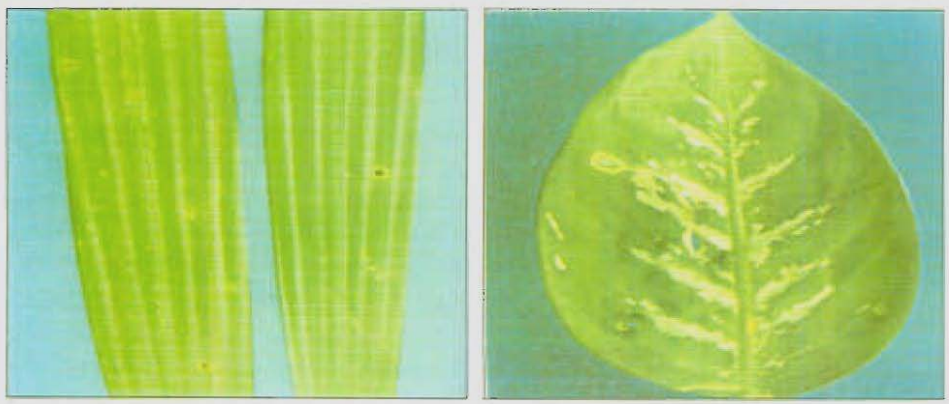

Fig. 5. Lesions caused by *Corynespora cassiicola* on leaves of dracaena (1) and dieffenbachia (2).

5-Host range:

Ten different foliage plant species were used to study their reaction to *C. cassiicola* isolates by using the detached leaves technique. The symptoms caused by isolates were observed two days after inoculation.

Data in Table (5) show that the tested plants varied in their reaction to different isolates. Dracaena isolate failed to induce symptoms on any tested plant species. While, dieffenbachia and pothos isolates gave symptoms on both *Ficus benjamina* and *F. microcarpa nitida* (cv. Hawaii), respectively. *Cissus rhombifolia* was moderately infected by only nephthytis isolate. No symptoms were noted on *Aglaonema commutatum, Asparagus densiflorus, Codiaeum variegatum, Philodendron cordatum, Schefflera actinophylla, Yucca elephantipes* and *Zamioculcas zamiifolia* by all the tested isolates.

Table 5. Reaction of ten foliage plant species to *C. cassiicola* isolates using detached leaf method

Tested host plant		Reaction of host plant to isolate*			
Common name	Scientific name	Dieffenbachia	Dracaena	Nephthytis	Pothos
Aglaonema	*Aglaonema commutatum*	-	-	-	-
Asparagus fern	*Asparagus densiflorus*	-	-	-	-
Croton	*Codiaeum variegatum*	-	-	-	-
Ficus	*Ficus microcarpa nitida* (cv. Hawaii)	++	-	-	+++
Grape Ivy	*Cissus rhombifolia*	-	-	++	-
Philodendron	*Philodendron cordatum*	-	-	-	-
Spineless yucca	*Yucca elephantipes*	-	-	-	-
Umbrella tree	*Schefflera actinophylla*	-	-	-	-
Weeping fig	*Ficus benjamina*	+	-	-	++
Zamioculcas	*Zamioculcas zamiifolia*	-	-	-	-

* Reaction: (-)= no infection, (+)= Slight infection, (++)= Moderate infection and (+++)= Severe infection.

6- Differentiation of the tested isolates by protein patterns obtained by sodium-dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS- PAGE) technique:

Protein of the four tested isolates of *C. cassiicola* were compared by SDS-PAGE. Photography of the separated bands of electrophoretic protein patterns are shown in Fig. (6) and their constructed phenogram based on similarity level generated from cluster analysis is show in Fig. (7).

The tested isolates formed two main clusters; the first cluster (97.81% similarity) consisted of isolates 3 and 4, isolated from nephthytis and dieffenbachia, respectively. While, the second one included isolates 1 and 2, isolated from pothos and dracaena, respectively, which recorded 94.58% similarity. However, the two clusters were closely related as they showed 89.72% similarity.

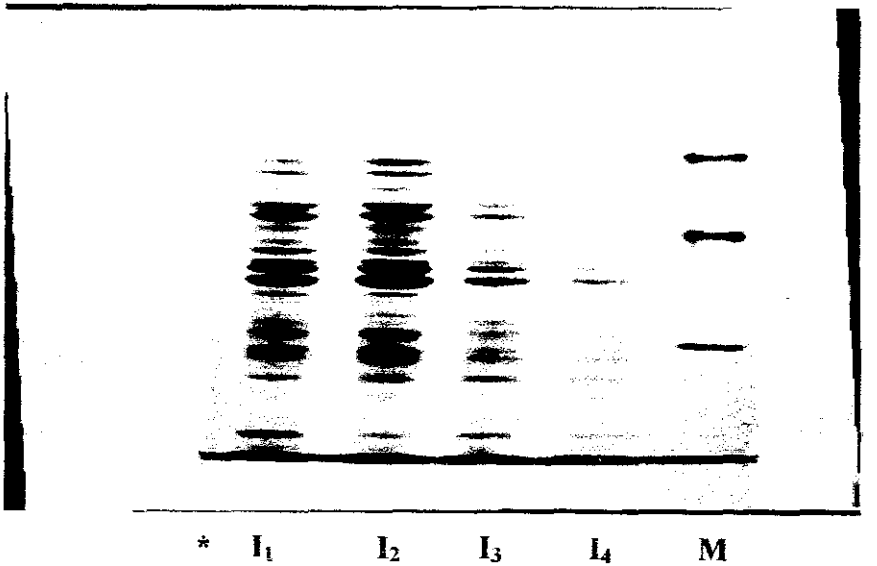

Fig.6. Photography of bands separated from the four tested isolates using 3 markers (M). Sizes of the protein marker fragments of 66.000, 48.000, 29.000 Kda.

* (I_1) isolated from pothos, (I_2) isolated from dracaena, (I_3) isolated from nephthytis and (I_4) isolated from dieffenbachia.

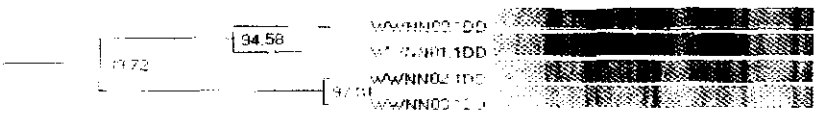

Fig. 7. Dendrogram showing protein pattern variations of the four *Corynespora cassiicola* isolates.

According to analysis of protein patterns illustrated in Fig. (8), separated protein bands were 18 for isolates 1and 2, 15 bands for isolate 3 and 12 bands for isolate 4. The four tested isolates of *C. cassiicola* showed complete similarity in the 8 separated bands at 61-62 kda, 51-52 kda, 43-44 kda,31-32 kda, 27-28 kda, 25-26 kda, 19-20 kda, and 17-18 kda.These 8 bands resembled 60.58%, 55.56%, 61.22% and 78.61% of the total protein separated from isolate 1, 2, 3 and 4, respectively. These specific eight bands may be characteristic for the genus Corynespora and require more studies to confirm that. Isolate 4 was characterized by missing four specific bands separated at 75-76 kda, 65-66 kda, 57-58 kda and 35-36 kda, although these particular bands were found in the three other isolates where they resembled 15.75%, 22.63% and 20.58% of the total separated protein,

Fig. 8. Number of separated bands, related molecular weight in peaks and percentage of area in the four tested *Corynespora cassiicola* isolates.

Molecular weight (Kda)	I_1 *		I_2		I_3		I_4	
	PK	%	PK	%	PK	%	PK	%
82-81	1	2.39	1	2.13				
80-79								
78-77								
76-75	2	2.69	2	2.80	1	4.63		
74-73	3	2.90	3	1.74				
72-71								
70-69								
68-67								
66-65	4	5.33	4	7.57	2	7.29		
64-63								
62-61	5	5.24	5	4.80	3	2.95	1	20.74
60-59								
58-57	6	2.46	6	2.96	4	4.26		
56-55								
54-53					5	6.10	2	5.49
52-51	7	10.37	7	14.54	6	8.37	3	7.89
50-49	8	3.89						
48-47								
46-45	9	2.86	8	3.29				
44-43	10	3.83	9	3.56	7	6.39	4	6.33
42-41	11	6.51			8	6.41	5	5.86
40-39			10	11.85	9	5.68	6	5.76
38-37	12	5.14					7	4.30
36-35	13	5.27	11	4.74	10	4.40		
34-33			12	2.81				
32-31	14	10.90	13	7.56	11	11.50	8	10.80
30-29								
28-27	15	9.33	14	9.85	12	9.53	9	9.51
26-25	16	9.19	15	4.74	13	10.68	10	10.08
24-23			16	4.56				
22-21								
20-19	17	8.59	17	7.36	14	7.90	11	8.78
18-17	18	3.13	18	3.15	15	3.90	12	4.48
		100		100		100		100

* Refer to footnote of Fig. (6) for explanation.

respectively. Isolate 1 was characterized by missing a specific protein band at of 39-40 kda, which was separated in the other isolates, where it resembled 11.85%, 5.68% and 5.76% of the total protein, respectively. Isolate 2 was characterized by missing a specific protein band at 41-42 kda, although it was separated in the other isolates, where it resembled 6.51%, 6.41% and 5.86% of the total separated protein, respectively. Isolate 1 was characterized by separated of one specific band at 49-50 kda where it resembled 3.89% of the total separated proteins. This particular band was completely missed in the other three isolates of C. *cassiicola*.

Isolate 2 also was more characterized by separation of two specific bands at 23-24 kda and 33-34 kda, where it resembled 4.56 and 2.81% of the total separated protein. These particular bands were completely missed in the other three isolates of *C. cassiicola*.

Discussion

Surveying nine ornamental foliage plants, *i.e.* aglaonema, grape ivy, croton, dieffenbachia, dracaena, nephthytis, philodendron, pothos and umbrella tree, in greenhouses of Giza governorate revealed 12 fungi associating with leaf spot symptoms. They were: *Alternaria panax*, *Asteromella* sp., *Botryodiplodia* spp. *Cephalosporium cinnamomeum*, *Colletotrichum* sp., *Corynespora cassiicola*, *Cylindrocarpon* sp., *Dactylaria humicola*, *Fusarium moniliforme*, *Myrothecium roridum*, *Nigrospora* sp. and *Rhizoctonia solani*.

The appearance of several leaf spot fungal diseases on these foliage plants caused by the aforementioned fungi was reported by Knauss *et al.* (1981), Blessington and Collins (1993), Chase *et al.* (1995) and Chase (1997).

According to the available literature, the recorded leaf spot diseases of the foliage plants tested and their fungal pathogens, except *F. moniliforme* on dracaena (Hilal *et al.*, 2000), are reported here in for the first time in Egypt(Ali *et al.*,1972 and Ziedan, 1980). *C. cassiicola* leaf spot was reported on 57, 69, 52 and 30 host plants by Ellis (1957), Ellis and Holliday (1971), Farr *et al.* (1989) and Chase *et al.* (1995), respectively. These hosts, however, included more than 50 ornamental plants as some of the foliage plants tested.

R. solani, *C. cassiicola*, *A. panax*, *Colletotrichum* sp., and *F. moniliforme* (12.9%) were the most dominant fungi on isolation, followed by *Myrothecium roridum* at low frequency. In this respect, Chase (1997) recorded *C. cassiicola* (on weeping fig), *Myrothecium* sp. (on aglaonema, dieffenbachia, ficus and nephthytis), *R. solani* and *A. panax* (on umbrella tree) and *F. moniliforme* (on dracaena plants). Regarding morphological features, the length of conidiophores and conidia as well as septum number per conidium were found to vary according to the host plant sources. Lengths were much longer in nephthytis isolate than in the other isolates and much shorter in dieffenbachia isolate than the others. Also, number of septa / conidium in nephthytis isolate was higher than in the other isolates. These variations, however, might be attributed to their genetic structures.

Regarding genetic variation there was 8 of similar molecular weight bands were separated from all the 4 investigated isolates. These specific eight bands may be characteristic for the genus Corynespora and require more studies to confirm that. However, each tested isolate was characterized by missing bands separated in the other ones. Moreover, Isolate 2 also was more characterized by separation of two specific bands at molecular weight of 23-24 kda and 33-34 kda, where it resembled 4.56 and 2.81% of the total separated proteins. These particular bands were completely missed in the other three isolates of *C. cassiicola* .Similar genetic variations were confirmed by Silva *et al.*, (1995); Silva *et al.*, (1998); Saha *et al.*, (2000) and Silva *et al.*, (2003).

Pathogenicity and cross-inoculation tests indicated that each of the four *C. cassiicola* isolates was pathogenic to the foliage plants tested, *i.e.* dracaena, dieffenbachia, nephthytis and pothos. Each isolate recording the highest disease index on its original plant. Dracaena isolate, however, was the most virulent on these plants, while that of dieffenbachia was the least aggressive one. In this respect variation in pathogenicity was observed by Duarte *et al.* (1983) among *C. cassiicola* isolates on cacao (*Theobroma cacao* L.) and papaya. On the other hand, studying the reaction of ten foliage plants, other than those of the pathogenicity tests, to the four *C. cassiicola* isolates confirmed that they greatly varied in this respect. The dracaena isolate failed to infect any tested plant species. While, only 5 reactions from 40 were positive in case of dieffenbachia and pothos isolates on weeping fig and ficus and that of nephthytis on only rape ivy. Variation in these plants susceptibility to the pathogen infection could be attributed to differences in their genetic make up and to different host pathogen interactions.

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تبقعات أوراق نباتات الزينة الورقية في مصر وخاصة المسبب *Corynespora cassiicola* [(Berk. & Curt.)Wei]

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تم حصر أهم مسببات أمراض تبقعات الأوراق التي تصيب تسع نباتات زينة ورقية مختلفة في العديد من المشاتل و النصوب بمحافظة الجيزة خلال عامي (1999و2000) . تم عزل 12 مسبب فطري من هذه النباتات بينما أختلف تواجد كل فطر من نبات لآخر.وكانت المسببات المرضية ريزوكتونيا سولاني و كورينيسبورا كاسيكولا والترناريا بانكس و كوليتوتريكم وفيوزاريوم مونيليفورم هم أكثر الفطريات شيوعا وانتشارا في العزل يتبعها ميروثيسيوم روريدوم. ولقد تم الحصول علي أكثر عدد من الأجناس الفطرية أثناء العزل من أربعة عوائل نباتية وهي :
Dracaena marginata, Epipremnum aureum, Philodendron cordatum, Syngonium podophyllum

وكان أكثر القطريات تكرارا في العزل الفطر *Corynespora cassiicola* علي نبات *Dieffenbachia seguine* وكذلك الفطرين *A. panax* و *R. solani* علي نبات *Schefflera actinophylla*. تم عمل قياس لطول وعرض كلا من الحوامل الكونيدية و الجراثيم الكونيدية لعزلات الفطر *Corynespora cassiicola* و أيضا عدد الحواجز العرضية لكل جرثومة كونيدية .

أثبت إختبار القدرة المرضية و العدوي العكسية أن العزلات الأربعة للفطر *Corynespora cassiicola* ممرضة لنباتات *Dracaena marginata, Dieffenbachia seguine, Epipremnum aureum, Syngonium podophyllum*

وسجلت أعلي إصابة علي النبات مصدر العزلة. يعتمد شكل أعراض الإصابة بتبقعات اوراق الفطر *C. cassiicola* بدرجة كبيرة علي العائل حيث تظهر أولا بقع دقيقة ؛ مائية تلونها بني فاتح قد تتسع مساحتها لتصل الي 2و5 سم و يدكن تلونها بتقدم الإصابة. كما أنه ممكن أن تتسع مساحتها بسرعة لتصل الي 5 سم أو أكثر عندما تكون الظروف البيئية مناسبة.

تم إثبات تعريف العزلات الأربعة لفطر *Corynespora cassiicola* بإستعمال طريقة التفريد الكهربي للبروتين والذي وجد تماثل نسبة 72و 89 % بين العزلات الأربعة والتي تكون مجموعتين رئيسيتين بكل منها عزلتين . المجموعة الأولي تضم العزلتين 3 ، 4 والتي تم عزلهم من نباتي *Syngonium podophyllum Dieffenbachia seguine* بنسبة تماثل 8و97% بينما سجل نسبة تماثل 3و 94% بين العزلتين 1،2 والتي تم عزلهم من نباتي *Epipremnum aureum, Dracaena marginata* بالمجموعة الثانية .

بإختبار المجال العوائلي للعزلات الأربعة علي عشر نباتات زينة ورقية بطريقة الورقة المفصولة إتضح وجود إختلاف كبير بين العزلات الأربعة. ولقد فشلت عزلة الدراسينا في إصابة أي من النباتات المختبرة بينما تم الحصول علي خمس تفاعلات موجبة من 40 حالة حيث أحدثت عزلتي *Dieffenbachia seguine* و *Epipremnum aureum* أصابة لكلا من نباتي *F. benjamina* و *Ficus microcarpanitida* (صنف هاواى) بينما عزلة *Syngonium podophyllum* أصابت نبات واحد فقط وهو *Cissus rhombifolia*. ولم تلاحظ أية أعراض إصابة لأي من العزلات الأربعة المختبرة علي نباتات *Aglaonema commutatum, Asparagus densiflorus, Codiaeum variegatum, Philodendron cordatum, Schefflera actinophylla, Yucca elephantipes, Zamioculcas zamiifolia*